



Sample Preparation of Urine/Plasma for Metabolomic Mass Spectrometry

Reagents needed:

- Methanol/Acetone – 90:10 (v/v), chilled to -20°C
- 10% Formic acid
- 5% Acetonitrile, 0.1% formic acid solution
- HPLC grade water (for blanks, and for preparing reagent solutions)
- Low-retention microcentrifuge tubes (e.g., Dot Scientific #RN1700-GST)
- Sample vials (e.g., Chromtech CPH-0952)

1. Pipet fresh samples of plasma or urine into 1.5 ml low retention microcentrifuge tubes, or thaw and resuspend aliquots of frozen plasma/urine samples. This protocol is outlined for 100- μ l volumes of urine or plasma per sample.

Note: include several water blanks carried through the entire extraction procedure (typically two blanks per set of 24 samples)

2. Add heavy-atom (deuterated or ^{13}C) standards to each sample (if desired).
3. Add 4 volumes (400 μ l) of chilled (-20°C) 90/10 methanol/acetone to each sample.
4. Vortex samples at high speed for 1 min. Samples may turn white as proteins denature and precipitate.
5. Incubate samples at -20°C for 15 min.
6. Centrifuge samples at 13,000 \times g, 15 min, 4°C. During this centrifugation step, label a new set of 1.5 ml microcentrifuge tubes (one for each sample).
7. Transfer the supernatants to the new microfuge tubes, avoiding the soft pellets.
8. Repeat the incubation at -20°C and centrifugation steps. Prepare and label a new set of 1.5 ml microcentrifuge tubes during the centrifugation step.

Note: the second incubation and centrifugation step is not necessary for urine samples.

9. Transfer the supernatants to the new microcentrifuge tubes.

10. Evaporate the samples to dryness under a stream of inert (nitrogen) gas.
11. Reconstitute
 - a. Urine samples in 75 ul of 5% acetonitrile, 0.1% formic acid
 - b. Plasma/serum samples in 100 ul of 5% acetonitrile, 0.1% formic acid
12. Vortex the samples to resuspend any particulates, and then centrifuge the samples at 13,000 x g, 5 min, 4°C.
13. Transfer the supernatants to MS vials containing the indicated volume of 10% formic acid:
 - a. Urine samples: 7 ul
 - b. Plasma/serum samples: 25 ul
14. Confirm that the sample pH < 3 by testing a microliter of the solution pipetted onto a pH indicator paper strip.
15. Store samples at -70°C or -80°C until used for mass spectrometry